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Evaluation of Some Plant Extract and Bio- Fungi to Control Leaf Spot of Safflower Plant Disease

LAYLA A. BENYAN

Department of Plant Protection, College of Agriculture, University of Basrah, Basrah, Iraq.

E- mail: Layla.Benyan@uobasrah.edu.iq

Abstract

This research study aims at evaluating the effectiveness of the biological fungus *Trichoderma longibrachiatum* , *Trichoderma harzianum* and some plant extracts kinocarpus*(Leucaena leucocephala)* ,Eucalyptus (Eucalyptus camaldulensis), henna leaf extract (*Lawsonia inermis)*. Besides, finding the best concentrations of plant extracts to stimulate the systemic resistance to safflower plant *Carthamus tinctorius, against alternaria leaf spot*. The results made clear that the fungus *Alternaria alternata* had clear effect in reducing the percentage of germination of safflower seeds as the rate reached 53.33% while the percentage of seedling damping off reached 81.11% compared with control treatment 93.33, 0 % respectively.

The biological fungi have high effectiveness with inhibiting growth of fungus *Alternaria alternata*, according to results as the antagonisim level was (1) as in bell scale. Plant extract also gave a high efficiency in preventing the infectious fungus's growth as rate of kinokarpus , henna, eucalyptus reached 75.78, 74.59, 70.64 % respectively. Effect of the extracts in inhibiting the pa thogenic funguswent up with increased concentration, as the highest percentage of inhibition was reached at a concentration of 10 % for tested extracts, the kinocarpus plant gave the highest rate reached 91.51%, while the percentage reached 89.62, 80.36 % for henna and eucalyptus extract, respectively.

Key words: plant extracts, safflower ,biofungi Trichoderma longibrachiatum , Trichoderma harzianum.

I. Introduction

The safflower crop *Carthamus tinctorius* L., that is a component of the Compositae family Astraceae which constitutes a significant oil crop. Economically, medically, because safflower oil is suitable for human use and contains a high percentage of essential unsaturated fatty acids, of which linoleic acid constitutes about 75%, which reduces the risk of atherosclerosis. Additionally, a variety of industries utilize its oil and vibrant flowers. such as soap and red , yellow dyes to color clothes and foods (Zhaomu and Lijie, 2001; Carvalho, et al. 2006)The remaining after oil extraction is rich in protein (about 24-40%) and is used for animals feeding (Oelike, et al. 1992). In several forms, the whole seeds may be used, mixed with barley to form food to feed dairy animals, which helps increase the percentage of fat and protein. The seeds are also used to feed poultry. The seeds may be grounded with or without the husk, and this flour is used to feed livestock. Seed flour with the husk is with Limited benefit for poultry because it contains a large percentage of fiber.

Safflower flour is incomplete in nutrition because it lacks some elements such as menothin. Safflower is one of the newly cultivated crops in Iraq, as its cultivation began during the year 1972-1973, and there are many attempts and experiments to cultivate it after overcoming some obstacles, especially since it is one of the crops with low water requirements compared to other oil crops that compete for the water share. It is also characterized by its tolerance to salinity and drought, which is suitable for the regions of central and southern Iraq, including Basrah Governorate. In addition to its seeds not being attacked by birds, safflower plants can export 65-92% of the dry matter collected before flowering to the seeds (Koutroubas et al., 2004).

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Exposure of these plants to moisture deficiency during the early growth stages led to a decrease in the number of heads and flowers in the plant, and seed yield was significantly associated with rainfall and minimum temperatures in the period between emergence and flowering (Saini and Westgate, 2000).

Safflower is infected with A wide range of fungal infections at various crop development phases, which are leaf spot (*Alternaria carthami*), fungus (*Alternaria alternata*), wilt (*Fusarium oxysporum* f. sp. *carthami*), root rot (*Phytophthora drechsleri*), rust (*Puccinia carthami*) and downy mildew. (*Bremia lactucae* f. sp. *Carthami*), (Girgis et al., 1989).

Among the many diseases reported on safflower, leaf spot caused by *A. carthami* is one of the most important types of blight. First reported by Chowdhury (1944) in Pune, India, the disease plays an important role in safflower cultivation and causes yield losses of 25 to 60% every year.

Aim of the study

Using some plant extracts and biological fungi to control leaf spot disease on safflower plants and finding the best concentrations to control the pathogen.

II. Materials and methods:

1-preparing food media for the purpose of growing and isolating fungi:

● Potato Dextrose Agar (PDA):

PDA medium was prepared with 20 gram of potatoes Then chopping them into little pieces before adding 500 cm3 of distilled water to boil them for 20 minutes in a glass flasks, then it was filtered. After that 20 gram of dextrose sugar and 17 gram of Agar was dissolved and completed the volume to a liter with distilled water, then distribute the medium into 250-ml glass flasks, which were closed with a cotton stopper and disinfected with an autoclave at a temperature of 121°C. It was left to cool and the antibiotic Chloramphenicol was added to it at an amount of 250 mg/liter before the medium solidified, then it was poured into petri dishes. The medium was manipulated to isolate pathogenic fungi and to conduct experiments to estimate the effect of plant extracts on the growth of pathogenic fungi.

● Water Agar medium (W.A):

This medium was made ready from agar (20 gm/L), one liter of distilled water, and 250 mg/L of the antibiotic Chloramphenicol added to it, as necessary, divide it into glass flasks. then sterilize in an autoclave .after Sterilization completed, The glass containers left and kept in the refrigerator. This medium was used to conduct pathogenicity experiments.

2. Isolation and diagnosis of the pathogen:

Samples of infected safflower leaves were collected from Basrah on 1/5/2022. They were placed in bags and brought to the laboratory. They were cut into pieces 0.5 cm long and used 10% sodium hypochlorate (from the 6% commercial solution) and sterilized with distilled for 2 minutes, then left to dry, and planted in 3 replicates of PDA petri dish , incubated for 4 days, then the fungi appeared in dish on the PDA medium were purified ,the fungi were identified based on their phenotypic and microscopic characteristics by preparing glass slides of the isolated fungi after adding lactophenol dye by A. M. Dr.. Yahya Ashour Saleh according to the taxonomic keys contained in (Ellis, 1971). The fungus were then stored in PDA medium in a slant refrigerator at 5° to the purpose of completing subsequent experiments (Ellis, 1971).

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Evaluating the pathogenic potential of isolated fungus on W.A medium:

Safflower seeds germination rate treated with the isolate of the fungus Alternaria was tested according to the method of Butler and Bolka (1974) using the nutrient medium W.A after it was sterilized with an Autoclave device and the antibiotic was added to it and shake well. After that, it was poured into Petri dishes with a diameter of 9 cm and left to solidify, then the center of each dish was exposed to a 0.5 cm-diameter fungal disc that was used as an inoculant. grown on PDA medium for a period of 7 days, then the plates were incubated for 3 days. Then, safflower seeds were superficially sterilized with a 6% sodium hypochlorate solution. The seeds were planted in a circular manner near the edge of the dish, with 10 seeds per dish at a rate of three repetitions, leaving a seed-containing treatment without any pathogenic fungi. and results were recorded after 7 days From planting to calculate the percentage of germination and seedling death as in the equation:

Germination percentage% =

Number of germinated seeds

---------- × 100%

Number of total seeds

Seedling death%=

Number of dead seedlings

---------- × 100%

Total number of seedlings

● **Preparing the alcoholic extract of the plants used in the experiment:**

The method (Jameela, 2011) was used , as 50 grams of dry plant leaf powder henna (*Lawsonia inermis*), kinocarpus , Eucalyptus, and placed it in 500 ml of 70% ethyl alcohol in a 1000 ml glass flasks and placed it in a sterile place for 7 days, then it was removed. Mix it well using a rotating magnet device and filter it using sterile gauze pieces to obtain the extract. It was filtered using a centrifuge device 3000 rpm for 10 min, left in dishes with a wide surface area to dry and get rid of the alcohol. The remaining extract was placed in test tubes until use.

● **The effect of alcoholic extract of the tested plants on the growth of pathogenic fungi:**

Prepare the PDA culture medium sterilized with an Autoclave device at a temperature of 121°C and a pressure of 15 pounds /inch^2 for 20 minutes. After the temperature decreases, the alcoholic extract is added to the medium at a concentration of 1%, 2%, 5%, and 10% for each extract. Then the glass flask is shaken for the purpose of homogenizing the extract with the culture medium then it was poured into petri dishes with a diameter of 9 cm and the dish was inoculated when the nutrient medium solidified with fungus discs, each with 0.5 cm of the growing medium on which the pathogenic fungus grew, with 3 duplicate for each. plates were incubated for 7 days and rate calculated for growth of fungal colonies, and inhibition percentage was calculated according to the equation:

_ Percentage of inhibition =

fungus growth rate in the comparison treatment – fungus growth rate in the control treatment

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fungus growth rate in the comparison treatment

● The effect of the fungi *Trichoderma harzianum* and the fungus *Trichodermalongibrachiatum* in pathologic fungi growth inhibition:

T. Longibrachiatum isolate was obtained in the Plant Protection Department, while the *T. harzianum* isolate was isolated from agricultural soil in Basrah. The *T. harzianum* fungus was diagnosed according to the classification key (Rifai, 1969), where the Bell (1982) method was used to measure the biological fungus ability in inhibiting the growth of pathogenic fungi, by place a disk of the dish containing a 5-day-old colony of pathogenic fungi near the edge of the dish, 1 cm away from the edge , facing it on the other side of the dish a disk of biological fungi was placed for 5 days in incubator , while control treatment left only the pathogenic fungus disc at a rate of 3 replicates for each treatment, the biological fungus growth measured by the proportion of inhibition is calculated by averaging two perpendicular diameters for every plate. As equation (Aboot ,1925) mentioned in Shaaban and Al-Mallah 1993).

$$\text{For inhibition} = \frac{R2 - R1}{R1} \times 100\%$$

Where R1 = maximum radiation growth of the pathogenic fungal colony in the comparison treatment

R2 = Maximum radioactive growth of the colony of pathogenic fungi in dishes containing biological fungus

● Testing the antibiotic efficacy as a bio resistance agent against pathogenic fungi in the laboratory:

The biological fungi ability was tested using the double culture approach. to combat pathogenic fungi. The petri dish containing the sterilized PDA culture medium was split into two equal sections. Next, a disk with a diameter of 0.5 cm from the edge of the colony of the biological fungus T. longibrachiatum at seven days old was inoculated in the center of the first section, spaced 1 cm from the dish's edge., Furthermore, a 0.5 cm diameter disk removed from the edge of the pathogenic fungal colony at 7 days old was used to inoculate the center of the second portion, which was 1 cm from the dish's edge.in the presence of a control treatment which was only exposed to the pathogenic fungus through an inoculation of a 0.5 cm diameter disk with 3 replicates for each treatment, then the dishes were incubated at 25 ± 2 C°. The degree of antagonism was calculated after the growth in the control treatment reached the edge of the dish according to the scale (Bell et al. 1982) consisting of 5 degrees, as follows:

1_ The whole plate is covered in biological fungus

2_ Over two thirds of the dish are biological fungus.

3_ 50% of the plate was covered with both biologic and pathogenic fungi

4_ The plate is two thirds covered in pathogenic fungus.

5_ the whole plate is covered with pathogenic fungi

An antagonism degree of 1 or 2 is deemed effective for the biological fungus

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Statistical analysis:

All laboratory experiments were conducted using a completely randomized design (CRD) for three- and two factors experiments. the percentage was angularly converted and compared the means according to the least significant difference method under the probability level of 0.01 in laboratory experiments

III. Discussion And Results :

1. Pathogenic fungi Identification and isolation :

The pathogenic fungus A. alternata was recognized based on its isolation and identification from safflower leaves based on Ellis (1971).

Figure 1. Diseased field of safflower at Basrah University, College of Agriculture

2. Testing the effect of fungal isolation on the percentage of germination and seedling death of safflower plants:

The results appeared in Table (1) and Figure (2) brought to light that the fungus had a clear effect on the percentage of germination of safflower seeds, with the rate reaching 53.33% for seed germination and 8.11% seedling death in the pathogenic fungus treatment compared to the control treatment of 93.33 and 0, respectively. This is due to the fact that *Alternaria* spp. fungi produce different toxins, whether specialized or non-specialized, that have the ability to spread from the site of infection to neighboring tissues, causing their death, which makes these tissues gradually diminish (Agarwal et al. 2001, Lemmlerg 1997). The *Alternaria alternata* fungus secretes low molecular weight toxins, including Tentoxin and Zinniol (Cupadhyay and Mukerji1997) . Microorganisms also have the ability to conjugate enzymes and growth regulators, so the pathogenicity of pathogens varies according to the mechanism of action of these metabolites either together or individually (Agrios, 2005)

Table 1 pathogenic fungi effect on safflower seedling mortality and germination percentage:

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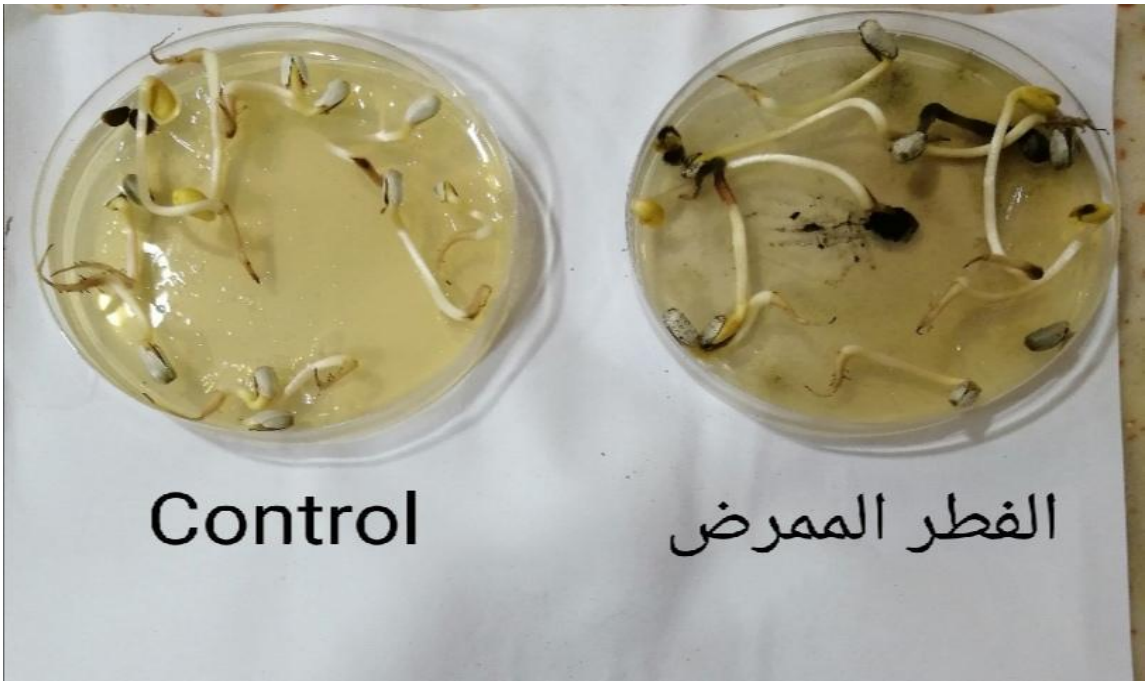
fungi	Germination %	Seedling mortality %
A. alternata	53.33%	81.11%
Control	93.33%	0
L.S.D	21.70	5.11

- Each number represents the average of three replicate



A B

Figure 2. Exhibited the pathogenicity of the fungus *Alternaria alternata* on germinating safflower seeds. A. Control B. *Alternaria alternata*

3-Testing of antibiotic efficacy as a biological resistance agent against the pathogenic fungus *Alternaria alternata* in the laboratory:

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Figure (3) results disclosed that *T. Longibrachiatum* and *T. harzianum* inhibit *A. alternate* development and the antagonism level obtained (1) for *T. Longibrachiatum*, which did not differ from the effectiveness of *T. harzianum* in inhibiting the fungus *A. alternata*, as the antagonism level obtained (1) as in the Bell (1982) scale. These results are in consistent with the findings of (Al-Abad, 2020) regarding the presence of a high antagonism ability for the fungus *T. Longibrachiatum* and pathogenic fungi. This ability is due to various mechanisms through which the fungus affects The pathogen is formed through using the biological fungus's mycelium to encircle the pathogenic fungus's threads to cause direct parasitism (Sivan and Chet, 1989), or It arises from the release of antibiotics and certain enzymes, including protease, B-1,3 glucanase, and chitinase, which break down the pathogenic fungus's cell walls. (Timon et al., 1999) or from Through the enjoyment of these mechanisms such as parasitism, antibiotics, and envelopment (Saad, 2001 and Kotasthane and Shalini, 2007)

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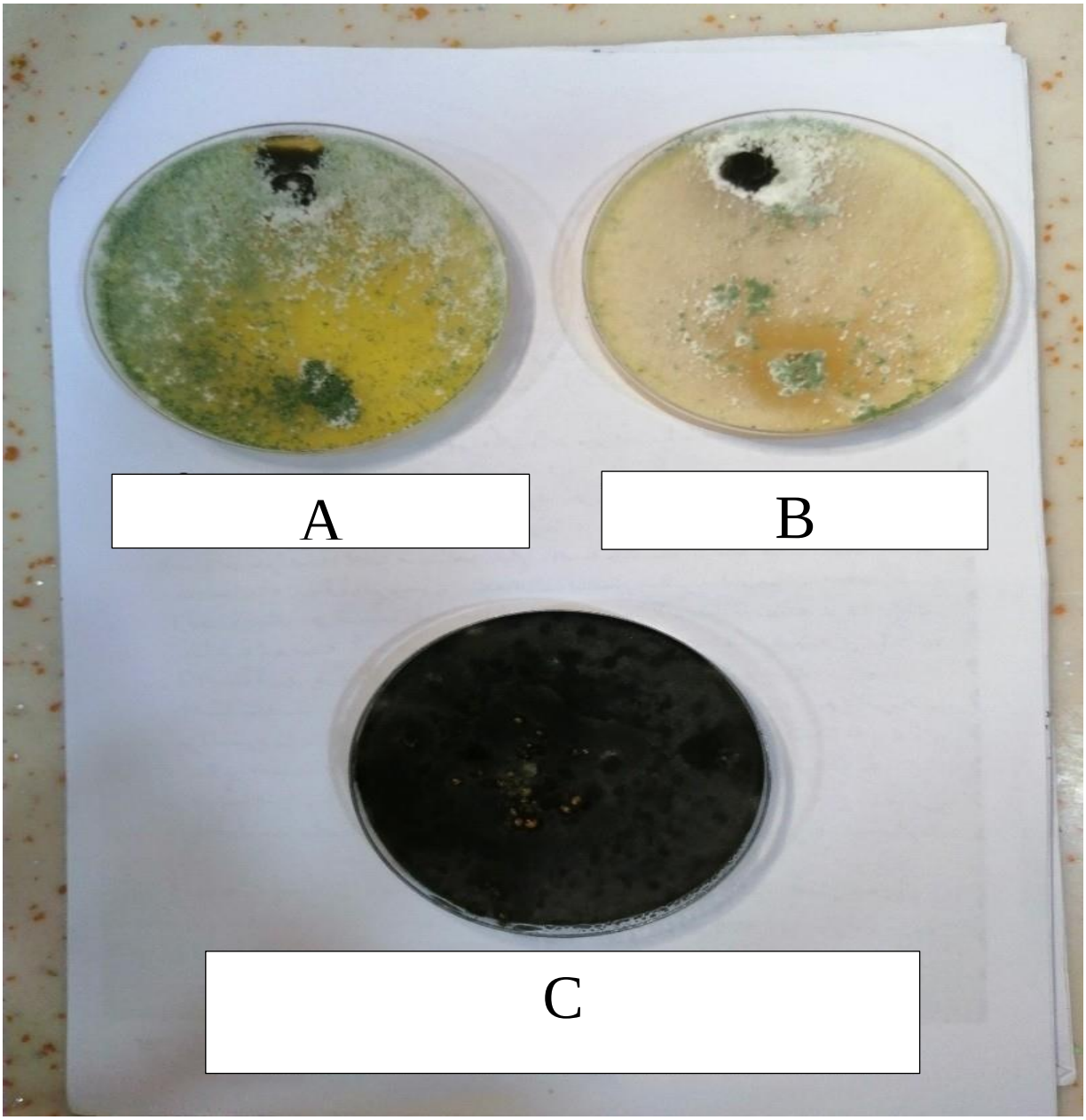

Figure 3. The effect of the biological fungi *Trichoderma harzianum* and the fungus *Trichoderma longibrachiatum* in inhibiting the growth of pathogenic fungi.

A. *T. harzianum* B. *T. longibrachiatum* C. Control.

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4. The effect of the alcoholic extract of the tested plants in inhibiting the growth of the fungus Alternaria alternata in the laboratory:

Results in **Table No. (2)** demonstrated the obvious influence that plant extracts have on preventing the pathogenic fungus growth ,as the average percentage of inhibition for kinocarpus, henna, and eucalyptus extracts reached 75.78, 74.59, and 70.64, respectively. The effect of the extracts in inhibiting the growth of the fungus *A. alternata* increased with increasing concentration, as the highest percentage of inhibition was reached at a concentration of 10% for the tested extracts. The highest percentage of inhibition was for kinocarpus and henna extract at a concentration of 10%, reaching 91.51 and 89.62%, with a significant difference from the eucalyptus extract, which amounted to 80.36. At a concentration of 10%, This was also evidently effective in preventing the pathogenic fungus growth ,because plant Henna, kinocarpus, and Eucalyptus contain phenolic substances, triterpenoids, and glycolipids. Al-Khair and Al-Obaidi (2020) attributed the susceptibility of Eucalyptus leaf extract and its effect on root rot fungi in pine seedlings to the fact that Eucalyptus leaves contain Flavonoids, Triterpenoids and Steroid, which have an effective role in inhibiting the growth of pathogenic fungi. Al-Adel and Aber (1979) also mentioned that the leaves, flowers and roots of many plants contain chemical compounds that have a toxic effect on agricultural pests When the infected plant parts decompose into the soil they release compounds that lead to the inhibition of plant pathogens and increase the effectiveness of microorganisms that have the ability to inhibit these pathogens, which increases the Control effectiveness (Goicoechea, et al. 2004).

Table (2) Depicts the effect of the alcoholic extract of the tested plants in preventing the pathogenic fungus Alternaria alternata growth in the laboratory:

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Effect rate Type of extract	Fungal growth inhibition % used Concentrations				extract
	10٪	5٪	2٪	1٪	
74.59	89.62	79.99	67.77	60.99	Lawsonia inermis
70.64	80.36	73.51	66.47	62.22	Eucalyptus
75.78	91.51	84.25	71.47	55.92	kinocarpus
	87.16	79.25	68.57	59.71	Average effect Concentrations
					L.S.D Extract 6.40 L.S.D concentration 7.39. L.S.D interference 12.8

The average of three replicates is characterized by each number.

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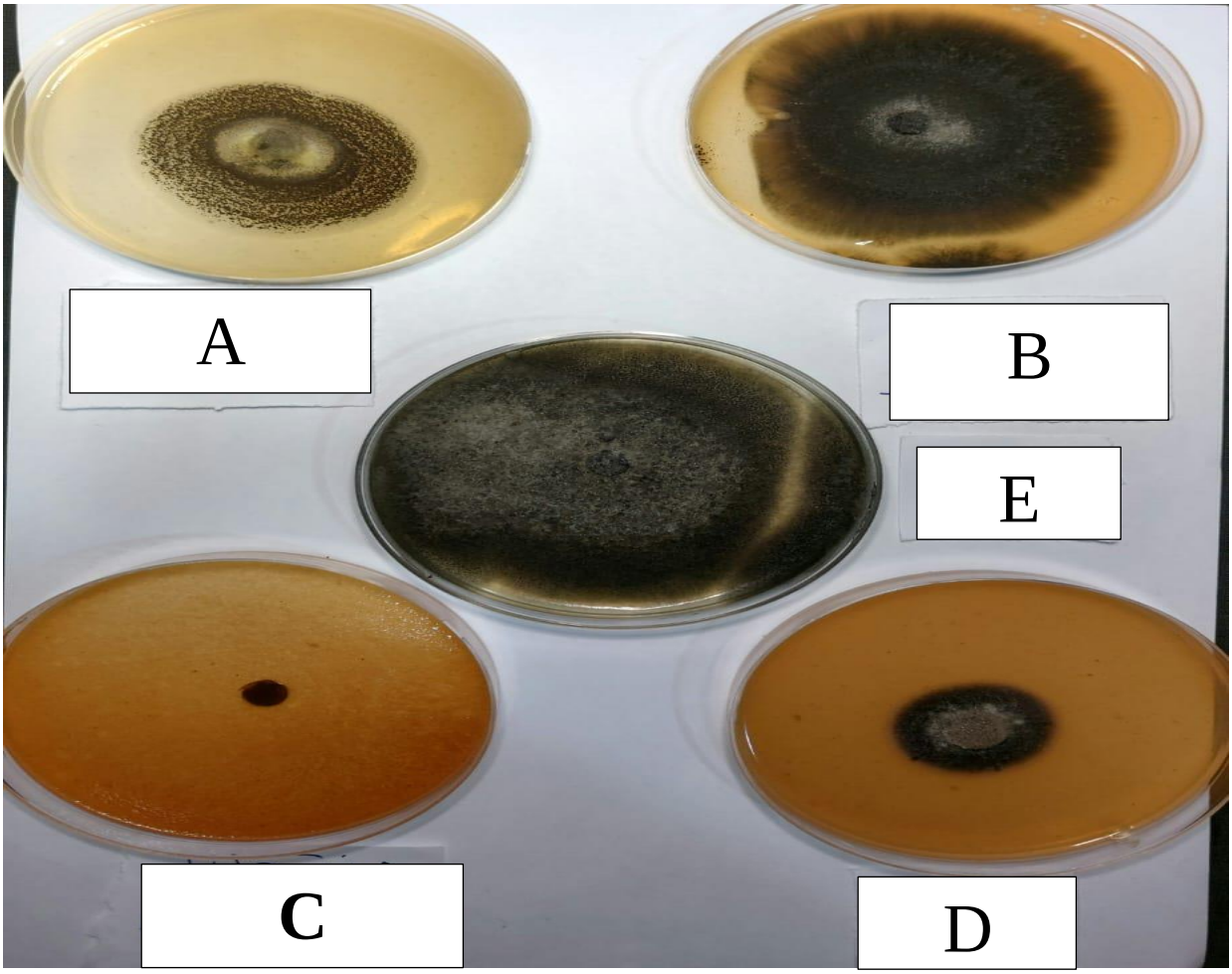

Figure 4. henna (*Lawsonia inermis*) plant extract effect

A. Henna 2% B. 1% C. 10% D. 5% E. control

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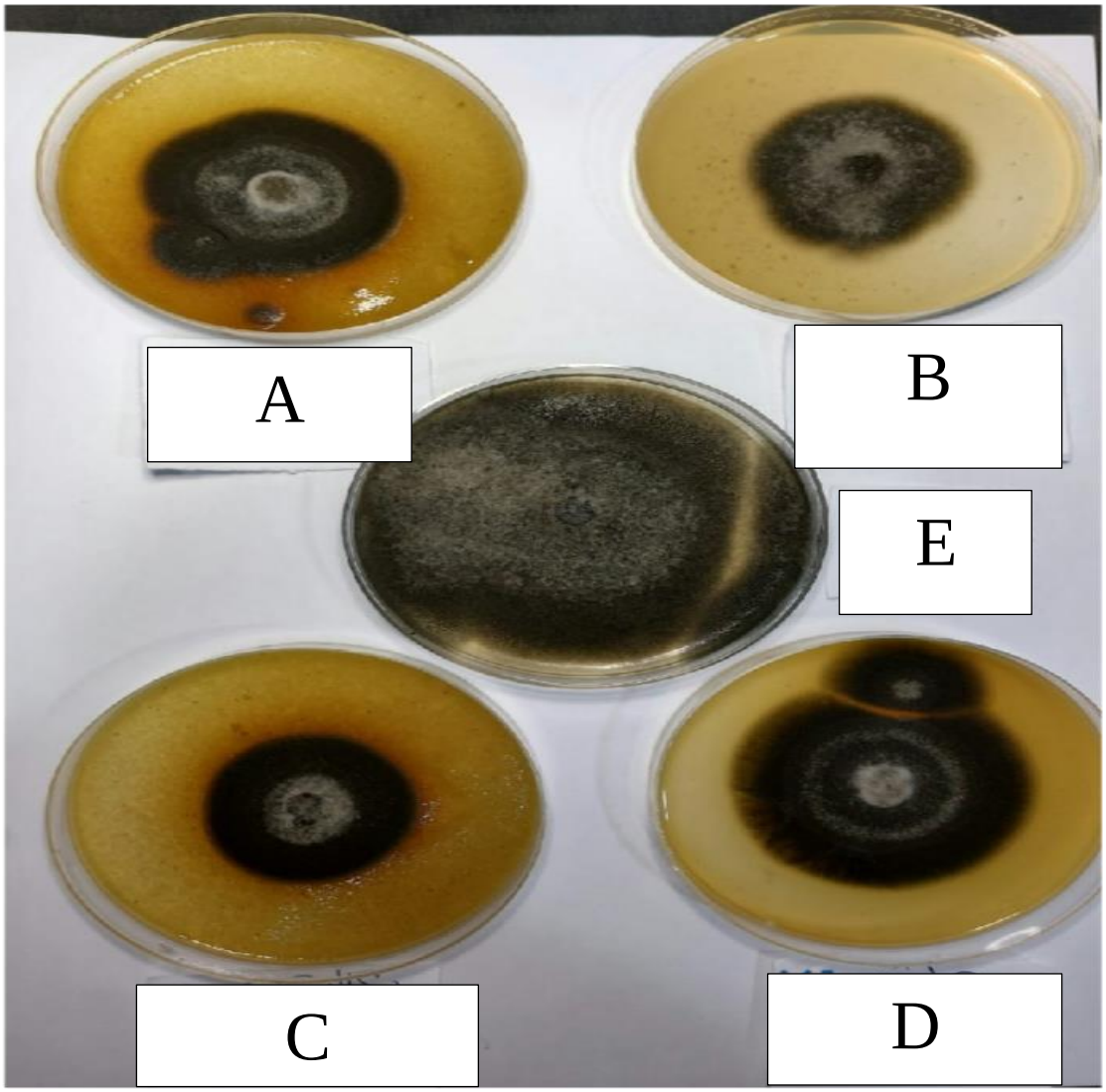

Figure 5. The effect of Eucalyptus extract

A. Eucalyptus extract 2% B. 5% C. 10% D. 1% E. control

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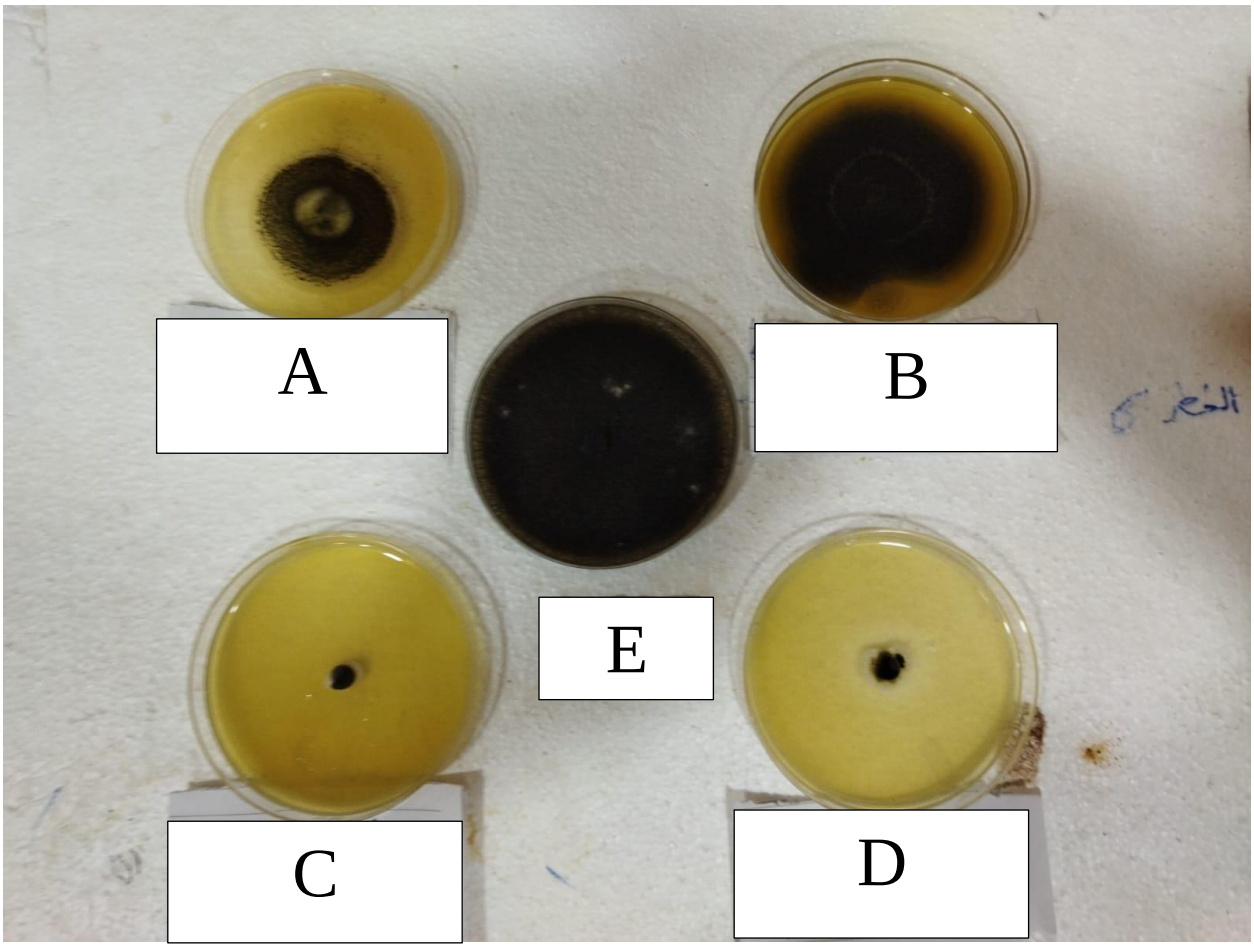

Figure 6. Illustrates the effect of kinocarpus for inhibion the growth of *Alternaria alternata*

A. Conocarpus 2% B. 1% C. 10% D. 5% E. Control.

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